IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Matthew Baker

Examiner: William O. Sandals, PhD

Application No.: 09/736,632

Art Unit: 1636

Filed: December 14, 2000

For: **ISOLATION OF NUCLEIC ACIDS**

DECLARATION UNDER 37 C.F.R. § 1.132

DECLARATION OF MATTHEW BAKER, PhD

1. I, Matthew Baker, am the Research Director of DNA Research Innovations Limited, the assignee of record of the above patent application. I am also the inventor of the above-mentioned patent application. A copy of my curriculum vitae is attached.

2. I have read the above-mentioned application, the Office Action dated April 9, 2003 and the references cited by the examiner, in particular, Journal of Chromatography, 37: 449-462, 1972 ("Kothari et al"), Biochemistry, 8(7): 2916-2923, 1969 ("Peterson et al") and Proc. Natl. Acad, Sci. USA, 69(8), 2317-2321, 1972 ("Reeck et al").

3. In the above-mentioned application, solid phases are disclosed which have a positive charge on at a first, lower pH that can be changed by varying the pH around the solid phase to make the surface less positive, neutral or negatively charged. In the application, these materials are referred to as "charge switch materials". As the backbone of nucleic acid is negatively charged, at the first lower pH at which the charge switch material is positively charged, nucleic acid will reversibly bind to the charge switch material, in preference to proteins and other contaminants typically present in a samples containing nucleic acid , e.g. culture broth, blood samples, or cell lysates. This allows the nucleic acid to be separated from such impurities, e.g. by simply washing them away in aqueous wash buffer. The nucleic acid sample can then be released from the solid phase under mild conditions, e.g. by

using a low salt buffer such as 10 mM Tris at pH 8.5. The method of the present invention provides nucleic acid samples in which the nucleic acid is not denatured, is present in a clean sample, is ready for use in standard molecular biology applications without the need for further processing. By way of example, the polymerase chain reaction (PCR) for amplifying nucleic acid is known to be sensitive to the presence of contaminating materials. Unlike many prior art protocols, the method of the invention provides a nucleic acid sample which is ready for PCR without further processing. A further advantage of the method of the present invention is that charge switch materials can be applied to almost any surface. This allows for development of purification protocols without format restriction, and opens up new possibilities for streamlining purification processes which have not been possible to date. Examples of surfaces that can be treated with charge switch materials: latex coated particles; glass and plastics, among others.

4. In the prior art, ion exchange purification is well known and have been used in the purification of different materials. It is important to appreciate that ion exchange columns and protocols employing them are different from the charge switch methods of the invention for the following reasons. In ion exchange chromatography, the pKa of the solid phase is selected so that the charge of the solid phase does not significantly change under the conditions in which the column is used for both binding and elution. This is because it is thought to be undesirable to have a ion exchange resin whose properties may change in use. This is contrary to what occurs in charge switch purification where the solid phase pK changes charge to bind and elute nucleic acid. As the ion exchange resins do not change their charge under conditions that would be used to purify nucleic acid, one result is that they bind nucleic acid very strongly. This makes it difficult to elute the nucleic acid without employing very high salt or pH extremes. Thus, rather than using a change in the charge of the column to elute a target material bound to the column, in ion exchange chromatography the target material is eluted by displacing the target material from the solid phase using ions, usually at high concentrations, which compete with the target material for binding to the column, thereby releasing it. This means that ion exchange chromatography presents particular disadvantages for the purification of nucleic acid as it binds strongly to the solid phase, often leading to damage to the nucleic acid when attempts are made to elute it at high pH . Also, the elution conditions used in ion exchange chromatography mean that the nucleic acid sample eluted from an ion exchange column is contaminated by high salt concentrations and other undesirable reagents. This means that the nucleic acid cannot be immediately used in

an application such as PCR, and instead requires further processing with the accompanying risk of damage to the nucleic acid in the sample.

5. The Peterson et al, Reeck et al and Kothari et al references describe a material known as ECTHAM cellulose which is prepared from epichlorohydrin and tris(hydroxymethyl)-aminomethane. In the cited references, this material is used for fractionating ribosomes and chromatin. I understand that the examiner has questioned whether ECTHAM cellulose has properties that would be suitable for use as a charge switch material for purifying nucleic acid.

6. In the experiments described in this declaration, I made a sample of ECTHAM cellulose and tested its properties in binding nucleic acid. These results were compared with those obtained in a corresponding experiment employing magnetic beads coated with one of the claimed charge switch materials, the polymerised biological buffer poly Bis-Tris (bis-hydroxyethyliminotrishydroxymethylmethane).

7. ECTHAM cellulose is not a commercially available material but can be made according to the procedure set out in the paragraph bridging pages 2916 and 2917 of Peterson et al. The protocol employed the following materials:

Tris (Trizma Base)	20g	Sigma T6066
NaOH	60g	Sigma S0899
Cellulose	60g	Sigma C6413 (column chromatography grade)
Epichlorohydrin	30ml	Sigma E1055
NaCl	500g	Sigma S9888
HCl	20ml	Sigma H7020
DNA	5mg	Sigma D1501

60g of NaOH was dissolved in 175ml of deionised water (dH2O) and chilled in an ice bath before adding the 20g of Tris. The Tris was dissolved and then 60g of cellulose added to form a crumbly solid. After thorough mixing with a glass rod and allowing to chill the mixture for about 30 minutes, the 30ml of Epichlorohydrin was added and mixed into the cellulose crumbs. The Epichlorohydrin was added in 10ml portions over about 15 minutes and any resultant heat over the next few hours was dissipated by placing the beaker in water

at room temperature. Following an overnight incubation, a 100ml portion of the mixture was removed and mixed with 500ml of 2M NaCl. This was then washed using a vacuum filter as described below:

500ml of 1M NaOH
500ml dH2O
500ml of 1M HCl
500ml of dH2O
500ml of 1M NaOH
500ml of dH2O

The ECTHAM cellulose was stored in 10mM Tris HCl at pH 8.5 with 20%v/v ethanol at around 50% v/v until employed in certain experiments conducted by Garry Harper, in which the pKa of a sample of the ECTHAM cellulose I prepared was measured. Those experiments are described in the declaration of Garry Harper which is being submitted concurrently with this declaration.

8. As stated in the above-mentioned declaration of Garry Harper, the commercial ion exchange resins DEAE Sepharose and DEAE Sephadex A50 were found to have pKa's of 14.16 and 14.23 respectively. These pKa values are very high as these materials are designed so that the charge of the solid phase does not significantly change under the conditions in which the column is used for both binding and elution. The experimentally determined pKa for ECTHAM cellulose was also very high at pKa 12.55. This material would therefore have similar properties to the ion exchanger resins. My interpretation of these results is that the chemistry used to produce ECTHAM cellulose using Epichlorohydrin has the effect of changing the pKa of the Tris component of ECTHAM cellulose from a pKa of 8.1 to a pKa of 12.55. I further conclude that ECTHAM cellulose has a pKa which is too high to make it useful as a charge switch material as described in the above mentioned patent application.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the

application or any patent issued thereon.

Declarant's signature 

Date: 23rd Sept. 2003

Curriculum vitae

Matthew Baker
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Date of birth: 16/12/58 **Married**

Employment history

June 1998

Technical Director, inventor and founder of DNA Research Innovations Ltd (DRI).
DRI was formed to exploit new technology for automated DNA extraction using a Charge Switch Technology (CST). DRI currently employs a staff of 25 of which 19 are full time scientists investigating DNA and RNA purification for the Medical, Food and Forensic markets. A team of five is dedicated to automation of CST formats and the development of a prototype instrumentation and consumables for clinical samples.
A small production department, ISO9001 accredited, manufactures and packs kits for a range of purification products.
A portfolio of 9 patents has been generated to cover all aspects of CST, magnetic solid phases and methods for new instrumentation and disposables.

Nov 1994 – June 1998

Self employed consultant specialising in the development of bioseparation methods for the molecular biology industry. A range of products were designed on a contract basis including new paramagnetic particles for DNA isolation, RNase assays for quality control purposes and latex particles for cell separation.

Sept 1991 – Sept 1994

Business Development manager for Scigen Ltd, a venture capital funded company involved with magnetic separation technology for Life Science research markets. Products were developed from composites of magnetite and celluloses derivatised with ion-exchangers. These were then formulated into DNA purification kits and marketed via distributors on an international basis.

Oct 1989 – July 1991

Business Development Manager for TFX Medical Ltd (Teleflex Inc). TFX is the medical arm of a US blue chip engineering company specialising in automative, aerospace and clinical markets. Production

facilities were set up in Ireland and the UK, producing OEM medical devices for intra venous, cardiac, epidural and gastroenterology applications.
Company growth was also achieved via acquisition of small and medium sized operations in complimentary fields.

Jan 1985 – 1989

Technical Sales specialist for Organon Teknika UK promoting anaesthetic drugs, haematology products and immunodiagnostic tests for the clinical markets.

Qualifications

1) B.Sc (hons) Biotechnology 2:1 Queen Elizabeth College, University of London

2) Master of Business Administration (MBA) Middlesex University, London.

Referees

Supplied on request